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TECHNICAL MANUSCRIPT 403

IDENTIFICATION OF CLONES OF CELLS
IN CULTURE BY DISC ELECTROPHORESIS

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IDENTIFICATION OF CLONES OF CELLS IN CULTURE
BY DISC ELECTROPHORESIS

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AGENT DEVELOPMENT AND ENGINEERING LABORATORY

Project 1C522301A059

August 1967

IDENTIFICATION OF CLONES OF CELLS IN CULTURE BY DISC ELECTROPHORESIS

ABSTRACT

Isoenzyme distribution patterns (zymograms) were obtained from various subclones of an L-Ma clone. On the basis of these zymograms, the various subclones were identified and classified. Preliminary experiments indicate that, although they can be modified by a change in the medium, the zymograms are reliable and useful criteria for identifying clones of cells in culture, when obtained from cells grown under identical and carefully controlled growth conditions.

Since the initial observation about 10 years ago that different proteins with similar enzyme activity existed in the same cell, organism, or tissue,¹⁻³ the study of isoenzymes and their distribution patterns has expanded into almost every area of biological research. Isoenzyme distribution patterns have been used for the clinical diagnosis of many diseases,⁴ the identification and differentiation of bacterial⁵ and animal¹ species, studies on developmental embryology,^{3,6} and for explaining control mechanisms in intermediary metabolism. Previous studies^{3,8,9} have concentrated on zymogram differences between the same tissues from different species or different tissues from the same species. The work presented here describes results obtained from various subclones of a cloned cell line in an attempt to systematize and correlate genotypic and phenotypic differences.

L-Ma C1-1 cells, a clone from the L-M cell line, were used for these studies. They were maintained in spinner culture on medium 199 peptone (199P) by a series of half-splits for 30 months after cloning and then recloned using a one-step dilution method as follows: from the spinners, 10 μ liter-samples were removed and mixed with 10 ml of 199P containing 20% horse serum, 50 units penicillin per ml, and 50 μ g streptomycin per ml. From this pool, 100 μ liters of the cell suspension were placed in Falcon tissue culture grade plastic petri dishes containing 10 ml of the medium containing 199P, 20% horse serum, and antibiotics. The monolayer was incubated in a 5% carbon dioxide atmosphere. At 4 weeks, 15 to 35 colonies per petri dish, representing a 15 to 35% plating efficiency, were observed, and, from these, clones were picked at random. The clones were first isolated by use of glass tubing (6 mm) wells, then aspirated from the surface of the petri dish with fresh cloning medium, and placed as monolayers in Falcon T-30 flasks.

The clones that were to be assayed for isoenzyme distribution patterns had been grown as monolayers in 199F with no serum for 52 to 71 passages. The cells were scraped from the glass with rubber policemen, packed by centrifugation, washed once with saline, resuspended in water, and disrupted by sonic oscillation in a Branson sonifier. The sample was placed at -20 C overnight and then thawed, and the supernatant liquid, after a single centrifugation at 4,000 x g for 10 minutes, was used for polyacrylamide disc electrophoresis by the method of Ornstein and Davis.¹⁰ The enzymatic activity was determined by standard assay. The cultures were all inoculated at the same concentration (4×10^5 cells per ml) and harvested at the same phase of growth (just as they formed a confluent monolayer). In addition, because the protein concentration varied by no more than twofold (0.14 to 0.24 mg per gel) among the various gels, we assumed that the amount of enzyme activity in any particular gel could be directly correlated with that of another gel.

The individual stained gels were scanned in a Canalco Model F microdensitometer equipped with an integrator, and the gels and tracings were examined to determine the peaks or bands of enzyme activity. The major bands of enzyme activity were numbered consecutively from the interface of the stacking and running gels. Various classes were established on the basis of the distribution patterns of the bands of enzyme activity. These are shown schematically in Figures 1 through 5.

Figure 1 illustrates the pattern of esterase isoenzyme activity using α -naphthyl acetate as substrate. The three classes are distinguished by the absence of bands 4 and 5 in Class 1, the absence of bands 1 and 7 in Class 2, and the presence of all bands except 1 in Class 3.

Figure 2 demonstrates the pattern of α -naphthyl propionate esterase activity. The three classes differ in that Class 1 contains no activity at bands 1 or 2, Class 2 contains activity at bands 1 and 2 but not at 3, and Class 3 shows activity at all positions except 2.

Figure 3 is the pattern when β -naphthyl acetate is used as a substrate for esterase. The three classes differ in that Class 1 contains enzyme activity only in bands 3 and 4, Class 2 only in band 3, and Class 3 only in bands 1 and 2.

Figure 4 is the isoenzyme distribution pattern of malic acid dehydrogenase. Class 1 contains activity only at band 2; Class 2 shows activity at the interphase and at band 1 and 2.

Figure 5 is the distribution obtained for DL-isocitric acid dehydrogenase. Here only two classes can be differentiated on the basis of the relative intensity of band 1 compared with that of band 2, using both visual observation and the activity measured by the microdensitometer.

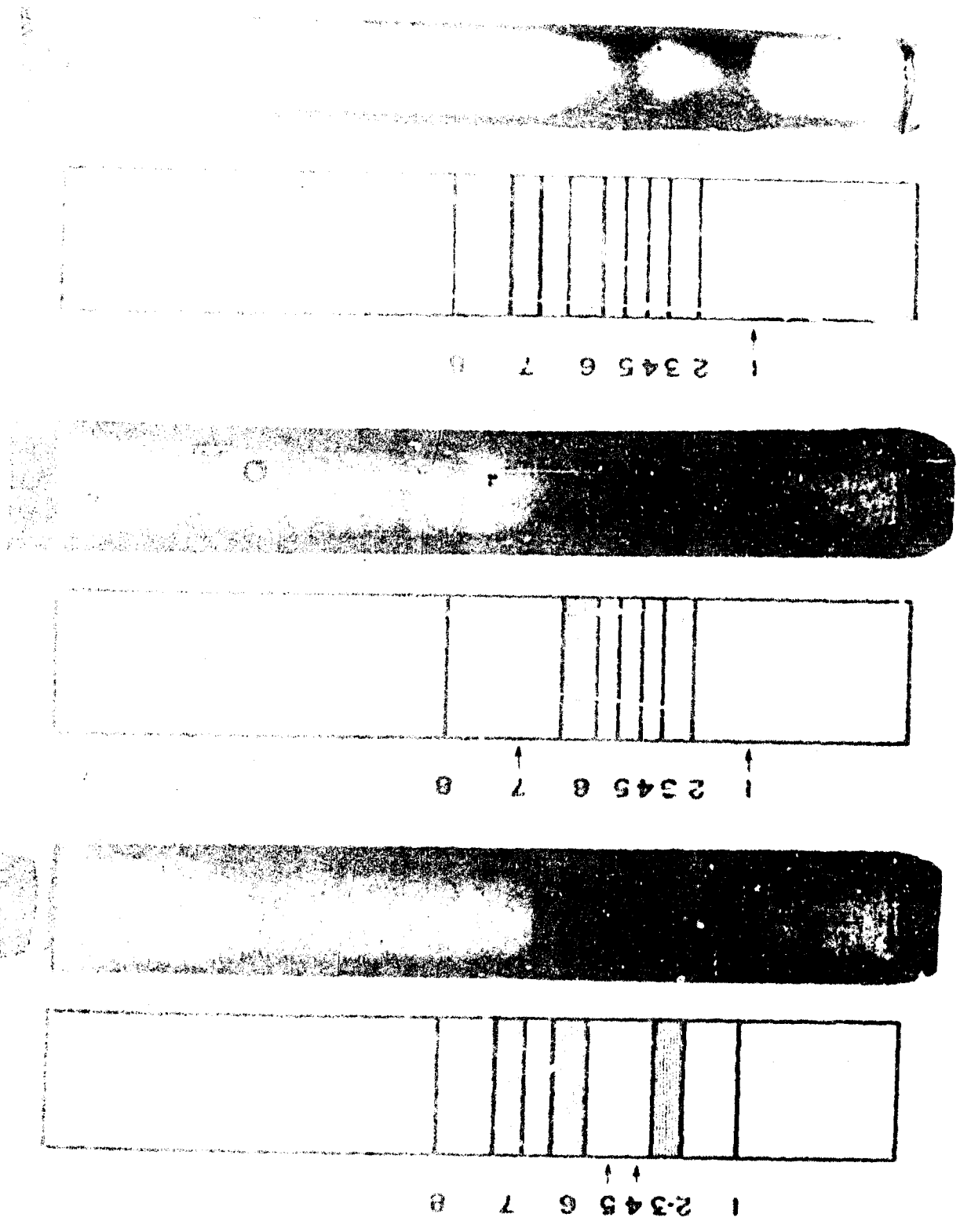


Figure 1. Isodensitometric Distribution Pattern for Isoenzyme Activity.

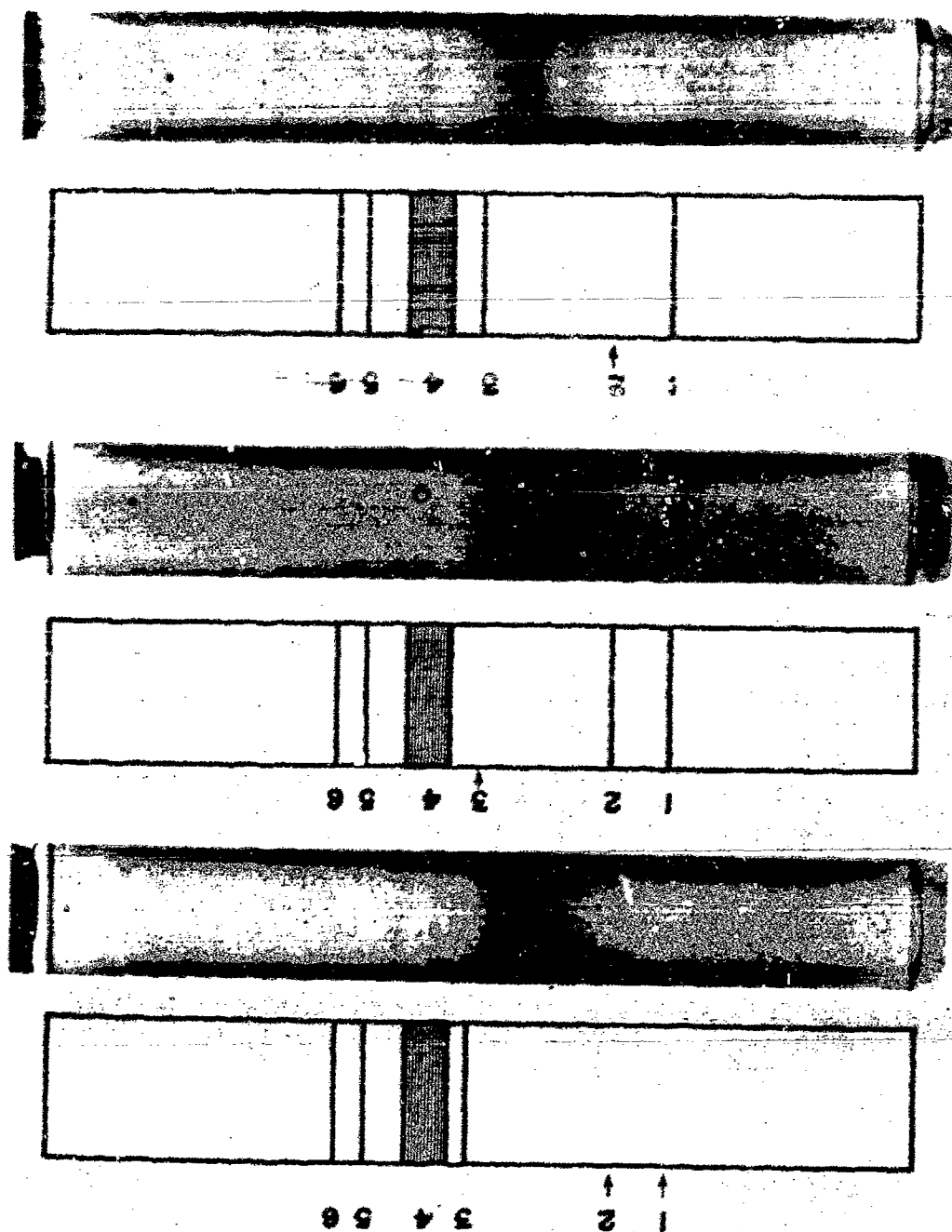
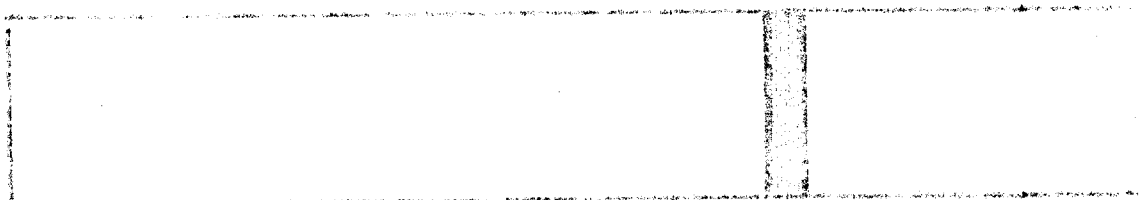
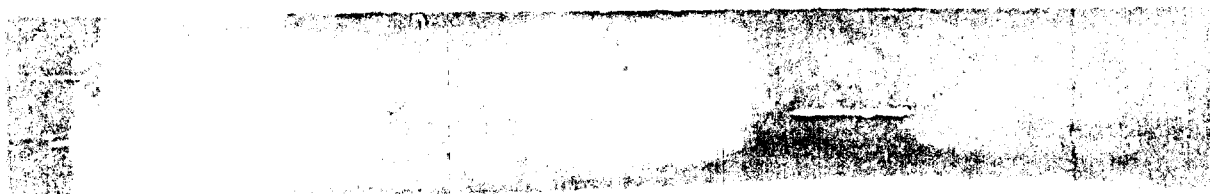


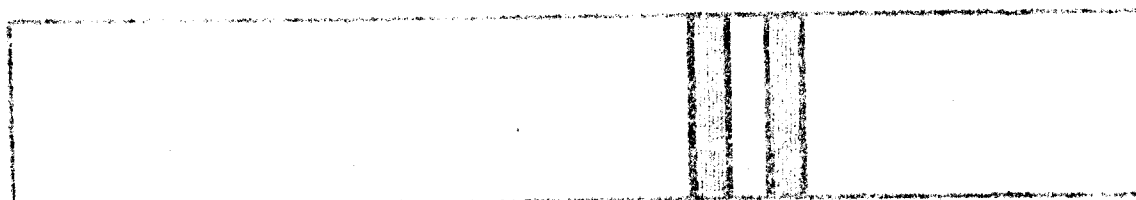
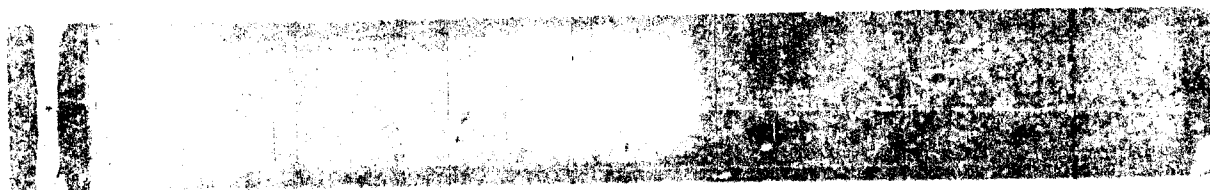
Figure 2. Isoenzyme Distribution Pattern for α -Naphthyl Propionate Esterase Activity.



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2 3 1



1
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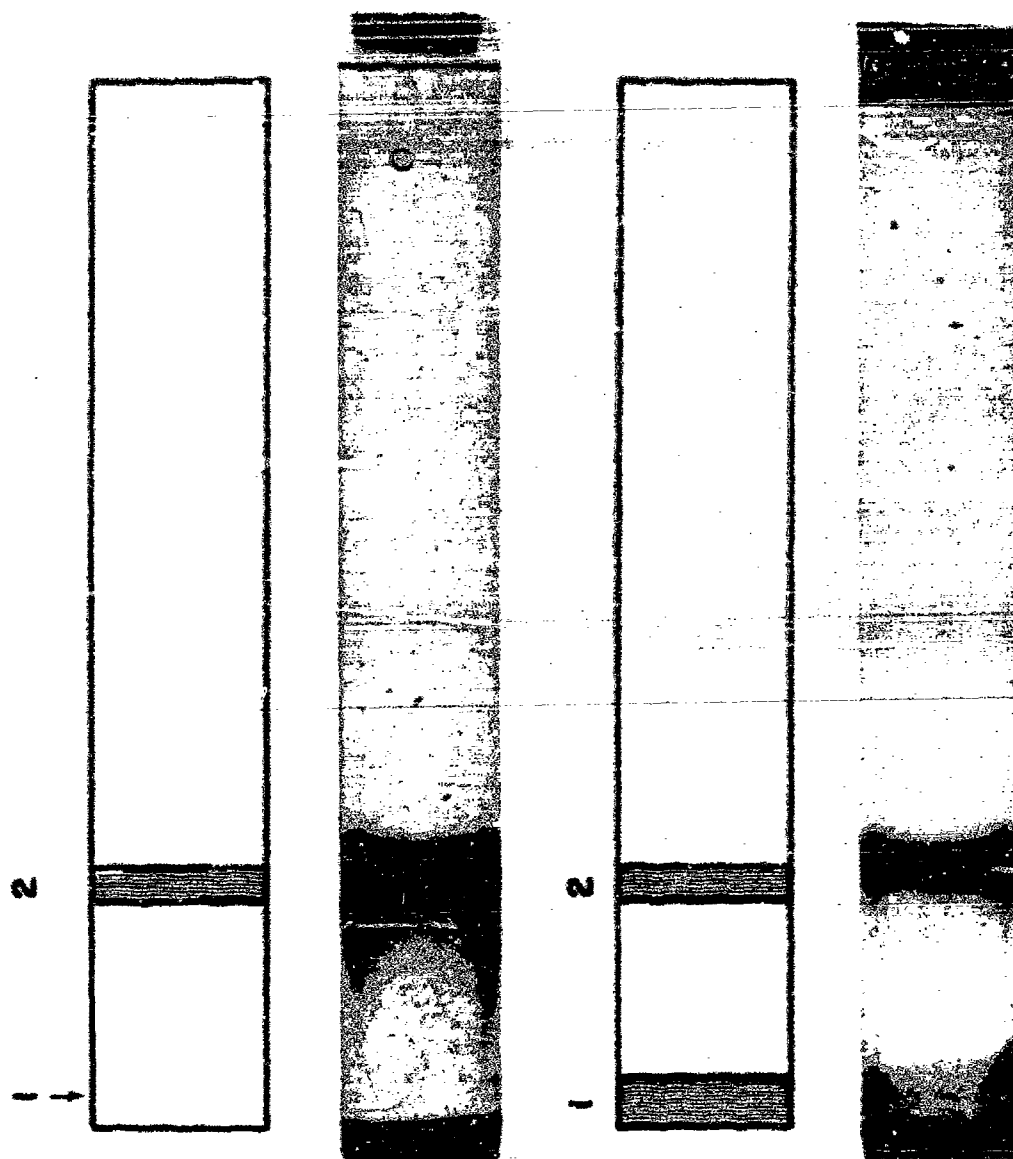


Figure 4. Isoenzyme Distribution Pattern for Malic Acid Dehydrogenase Activity.

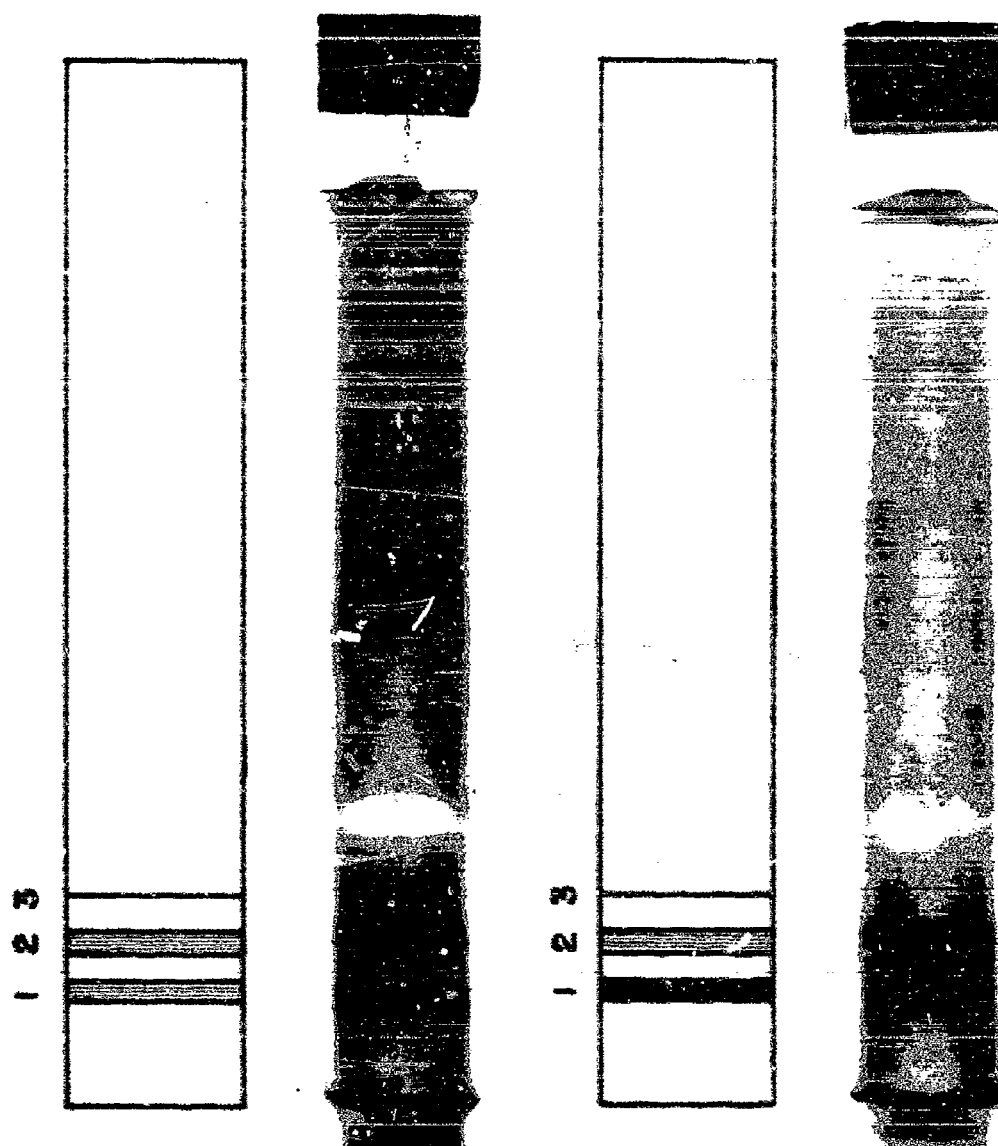


Figure 5. Isoenzyme Distribution Pattern for DL-Isocitric Acid Dehydrogenase Activity.

No differences were found in the symograms of lactic acid, glucose-6-phosphate, α -hydroxy butyrate, β -hydroxy butyrate, α -hydroxy phenyl-propionate, or succinic acid dehydrogenases or of α -naphthyl stearate esterase. We have been unable to demonstrate the presence of either acid or alkaline phosphatase activity in the gels. There was some indication of differences in the protein stains but the interpretation of these bands is too complicated to present here.

The clones were grouped and classified according to differences in their isoenzyme distribution patterns. The four clones studied in greatest detail, each assayed a minimum of six times, are characterized in Table 1. These are subclones of L-Ma Cl-1 that produce a large compact colony when cloned and exhibit similar growth characteristics. However, as is readily seen in Table 1, they can be distinguished by their isoenzyme distribution patterns.

TABLE 1. ISOENZYME DISTRIBUTION IN SUBCLONES OF L-Ma Cl-1

Isoenzyme Substrate	Class of Isoenzyme Distribution Pattern				
	Parent	Subclones			
	Clone 1	1-7	1-9	1-2	1-3
Esterase (α -naphthyl acetate)	1	1	1	2	3
Esterase (α -naphthyl propionate)	2	2	1	1	3
Esterase (β -naphthyl acetate)	1	3	1	2	3
Dehydrogenase (malic acid)	2	2	1	2	2
Dehydrogenase (DL-isocitric acid)	2	2	1	2	2

Subclone 1-7 is the same as the parent clone except for its pattern on β -naphthyl acetate. Subclone 1-9 differs from the parent in one of the esterases and from subclone 1-7 in two of the esterases. In addition, it varies from both the parent and 1-7 for malic acid dehydrogenase and DL-isocitric acid dehydrogenase. Subclones 1-2 and 1-3 also can be identified by the use of several of the isoenzyme substrate systems. These zymograms can readily be obtained, and by their use the various clones can be distinguished.

On the basis of these data, we conclude that it is possible to identify clones of mammalian cells grown in culture by their isoenzyme distribution patterns when grown under identical conditions. Preliminary work in our laboratory suggests that growth in different media and in monolayer vs. suspension results in a shift in the symogram, but that when grown under identical conditions the patterns are reproducible. This study further emphasizes the necessity for constant and carefully controlled conditions for cell growth if meaningful data are to be collected.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the source report is classified)

1. ORIGINATING ACTIVITY (Corporate author)		2a. REPORT SECURITY CLASSIFICATION	
Department of the Army Fort Detrick, Frederick, Maryland, 21701		Unclassified	
3. REPORT TITLE		2b. GROUP	
IDENTIFICATION OF CLONES OF CELLS IN CULTURE BY DISC ELECTROPHORESIS			
4. DESCRIPTIVE NOTES (Type of report and inclusive dates)			
5. AUTHOR(S) (First name, middle initial, last name)			
Fish, Donald C. Walker, Jerry S. Dobbs, James P. Carter, Richard C.			
6. REPORT DATE		7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
August 1967		14	10
8a. CONTRACT OR GRANT NO.		8b. ORIGINATOR'S REPORT NUMBER(S)	
A. PROJECT NO. IC522301A059		Technical Manuscript 403	
c.		9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)	
4.			
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11. SUPPLEMENTARY NOTES		12. SPONSORING MILITARY ACTIVITY	
		Department of the Army Fort Detrick, Frederick, Maryland, 21701	
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14. Key Words *Clones *Tissue cultures *Electrophoresis Enzymes Dehydrogenases Esterase			

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